30 YEARS OF THE MINERALOCORTICOID RECEPTOR

Mineralocorticoid receptor mutations

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Abstract

Aldosterone and the mineralocorticoid receptor (MR) are key elements for maintaining fluid and electrolyte homeostasis as well as regulation of blood pressure. Loss-of-function mutations of the MR are responsible for renal pseudohypoaldosteronism type 1 (PHA1), a rare disease of mineralocorticoid resistance presenting in the newborn with weight loss, failure to thrive, vomiting and dehydration, associated with hyperkalemia and metabolic acidosis, despite extremely elevated levels of plasma renin and aldosterone. In contrast, a MR gain-of-function mutation has been associated with a familial form of inherited mineralocorticoid hypertension exacerbated by pregnancy. In addition to rare variants, frequent functional single nucleotide polymorphisms of the MR are associated with salt sensitivity, blood pressure, stress response and depression in the general population. This review will summarize our knowledge on MR mutations in PHA1, reporting our experience on the genetic diagnosis in a large number of patients performed in the last 10 years at a national reference center for the disease. We will also discuss the influence of rare MR variants on blood pressure and salt sensitivity as well as on stress and cognitive functions in the general population.

Key Words

- ▶ mineralocorticoid receptor
- ▶ pseudohypoaldosteronism type 1- PHA1
- ▶ aldosterone
- ▶ hormone receptors
- ▶ nuclear receptor

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Introduction

Aldosterone and the mineralocorticoid receptor (MR) play a key role in the regulation of electrolyte balance and blood pressure. Abnormalities in aldosterone and MR function lead to salt-losing disorders or hypertension. Aldosterone is synthesized in the zona glomerulosa of the adrenal cortex under the control of different stimuli, mainly the renin-angiotensin system and potassium. Aldosterone regulates transepithelial ionic transport by stimulating sodium reabsorption and potassium secretion in the distal tubule of the kidney, the colon, salivary and sweat glands via binding to the MR (Pearce *et al.* 2003). The MR is a member of the steroid hormone receptor subgroup of the nuclear receptor superfamily (Nuclear Receptor Committee 1999) and acts as a ligand-dependent

transcription factor by binding to specific hormone-responsive elements located in regulatory regions of target genes. In the distal nephron, the MR induces an aldosterone-dependent dynamic transcriptional program, leading to rapid induction of different signaling pathways and transcriptional cascades modulating the activity of major structural components of ion transport. In the aldosterone-sensitive distal nephron, aldosterone regulates sodium and potassium balance by stimulating the activity of the epithelial sodium channel ENaC and the Na⁺-K⁺-ATPase (Stockand 2002). The MR rapidly induces transcription of the serum- and glucocorticoid-regulated kinase (sgk) 1, which directly stimulates ENaC activity by phosphorylating Nedd4-2, thus reducing

ubiquitylation, retrieval and degradation of ENaC (Bhalla *et al.* 2006). In parallel, transcriptional regulation of the deubiquitylating enzyme Usp2-45 increases ENaC surface expression and activity (Verrey *et al.* 2008). MR also increases the expression of glucocorticoid-induced leucine zipper protein (GILZ), which acts in parallel with sgk1 to increase ENaC localization at the plasma membrane (Soundararajan *et al.* 2005).

In addition to its effects on the kidney, a large number of studies have highlighted the role of the MR in regulating the physiological processes in non-epithelial tissues, including the heart, vessels, adipose tissue and the brain (Jaisser & Farman 2016).

It is important to remember that MR possesses the same affinity for aldosterone and for the physiological glucocorticoid cortisol (corticosterone in rats and mice), which has plasma concentrations 100- to 1000-fold higher than those of aldosterone. In aldosterone target tissues, the enzyme 11-beta-hydroxysteroid dehydrogenase type 2 (11HSD2) converts cortisol (and corticosterone) into the inactive metabolites cortisone and 11-dehydrocorticosterone, thus modulating intracellular glucocorticoid levels and protecting the nonselective MR

from occupation by glucocorticoids (Edwards *et al.* 1988, Funder *et al.* 1988 for a detailed review, see the article by Funder in this issue). In addition to local metabolism of the glucocorticoid hormones, intrinsic properties of the MR, in terms of ligand discrimination and coregulator recruitment, contribute to modulate receptor selectivity and transcriptional effects (Farman & Rafestin-Oblin 2001). In unprotected tissues, like adipose tissue and the brain, MR is a high-affinity receptor for glucocorticoids; however, depending on the cell type and environmental conditions, glucocorticoids may have agonist or antagonist effects (Funder 2005).

The study of MR variants has contributed enormously to our understanding of structure–function relationships in the MR. Frequent genetic variants of the MR are associated with salt sensitivity, blood pressure, stress response and depression. In extreme cases, loss-of-function mutations are responsible for renal pseudohypoaldosteronism type 1 (PHA1), whereas a gain-of-function mutation has been associated with a familial form of inherited mineralocorticoid hypertension. More than 380 rare coding variants have been described in the major transcript of the *NR3C2* gene, coding for the MR,

Figure 1

Three-dimensional homology model of the MR LBD. (A) Overall structure of the MR LBD, α -helices are drawn as ribbons and β -strands as arrows. Aldosterone is inserted into the ligand-binding pocket (carbon atoms are in white and oxygen atoms in red). (B) Linear scheme indicating amino acids of the MR LBD-contacting aldosterone. Hydrogen bonds are depicted as arrows, van der Waals contacts as dashed lines. W, water molecule. Reprinted from *Molecular and Cellular Endocrinology*, Volume 350, Huyet J, Pinon GM, Fay MR, Rafestin-Oblin ME & Fagart J, Structural determinants of ligand binding to the mineralocorticoid receptor, pages 187–195. Copyright 2012, with permission from Elsevier.

in the Exome Aggregation Consortium (ExAC) (Lek *et al.* 2016), which aggregates exome sequencing data from a wide variety of sequencing projects, with constraint metrics indicating that *NR3C2* is intolerant for both missense and nonsense mutations. This suggests that rare variants (MAF less than 0.5%) and also common single-nucleotide polymorphism, with a minor allele frequency (MAF) in the population $\geq 5\%$, may have functional consequences on mineralocorticoid homeostasis in the general population and modulate disease susceptibility whenever they significantly affect protein function or amount.

Pseudohypoaldosteronism type 1

The integrity of the mineralocorticoid axis is particularly relevant in the neonatal period, where renal regulation of water and electrolyte balance is impaired due to immature tubular function (Holtback & Aperia 2003). Events such as prematurity or infections may lead to salt loss and dehydration, which can be further amplified by physiological partial resistance to aldosterone in the newborn (Martinierie *et al.* 2009). PHA1 is a rare disease of mineralocorticoid resistance, first described by Cheek and Perry in the early 50s of the last century (Cheek & Perry 1958). The disease presents as a salt-wasting syndrome in the neonatal period, with weight loss, failure to thrive, vomiting and dehydration, associated with hyperkalemia and metabolic acidosis, despite extremely elevated levels of plasma renin and aldosterone (Zennaro *et al.* 2012). Seminal work by Armanini and coworkers and Kuhnle and coworkers has characterized the clinical and pathogenic mechanisms of PHA1 and paved the way to the discovery of the underlying genetic abnormalities, showing abnormalities of aldosterone binding on mononuclear leukocytes from patients with PHA1 and different transmission of the hormonal and binding defects (Armanini *et al.* 1985, Kuhnle *et al.* 1990). These results were further confirmed by clinical studies describing the existence of two distinct clinical and genetic entities associated with different severity and evolution: a renal form, in which signs of mineralocorticoid resistance are restricted to the kidney and a generalized form, where systemic mineralocorticoid resistance in the kidney, but also in the colon, salivary and sweat glands and the lungs, leads to severe salt loss from multiple organs (Hanukoglu 1991).

Renal PHA1 (also called autosomal dominant PHA1, registered in the online repositories OMIM (<https://www.omim.org/>) and Orphanet (<http://www.orpha.net/>) under

numbers MIM#177735 and ORPHA#71871) is a mild form of primary mineralocorticoid resistance and represents the most frequent form of the disease. It is due to loss-of-function mutations in the *NR3C2* gene (Geller *et al.* 1998, Sartorato *et al.* 2003, Pujo *et al.* 2007). The prevalence, as estimated from recruitment through a national reference center for rare diseases (MC. Zennaro, genetics laboratory, HEGP, see below) is ~ 1 per 80,000 newborns, but may be underestimated due to phenotypic variability, including asymptomatic cases. Patients show various degrees of failure to thrive, dehydration and vomiting, with hyponatremia, hyperkalemia and inappropriately high urinary sodium excretion. In contrast, urinary potassium excretion is low, with reduced fractional potassium excretion and transtubular potassium gradient (Rodriguez-Soriano *et al.* 1990). Diagnosis is confirmed by elevated plasma renin and aldosterone levels. Symptoms of renal PHA1 improve in childhood and, generally, salt supplementation, which is required to correct the sodium losses, can be discontinued around age 18–24 months. Older children are generally clinically asymptomatic. Nevertheless, a recent case-control study investigating 39 adult patients with renal PHA1 carriers of *NR3C2* mutations showed that they show lifelong increased plasma renin and aldosterone levels as well as increased salt appetite, with normal blood pressure and potassium levels (Escoubet *et al.* 2013), confirming evidence from earlier case reports suggesting persistence of hormonal abnormalities in adulthood (Kuhnle *et al.* 1990, Zennaro *et al.* 1992, Geller *et al.* 2006). Remarkably, high aldosterone levels and salt intake in the context of low MR activity are not associated with adverse cardiovascular outcome in these patients, but rather with improved diastolic left ventricular function (Escoubet *et al.* 2013).

In contrast to the renal form, generalized PHA1 (MIM #264350, ORPHA#171876, also called autosomal recessive PHA1) is a severe salt-wasting syndrome with profound hyponatremia and hyperkalemia, which may be complicated by cardiac dysrhythmias, collapse, shock or cardiac arrest (Speiser *et al.* 1986). Salivary or sweat tests are positive, and a subset of patients show respiratory tract illnesses (Kerem *et al.* 1999) and cutaneous lesions similar to those appearing in miliaria rubra (Belot *et al.* 2008). Generalized PHA1 is due to loss-of-function mutations in one of the three genes coding for the subunits of the epithelial sodium channel ENaC (*SCNN1A*, *SCNN1B* and *SCNN1G*) (Chang *et al.* 1996, Strautnieks *et al.* 1996). Early diagnosis of the disease is critical to survival, and prognosis is poor with no remission reported. Patients experience recurrent life-threatening episodes of salt loss, requiring

life-long supplementation with high doses of sodium and ion-exchange resins (Belot *et al.* 2008, Hanukoglu & Hanukoglu 2010).

MR mutations in PHA1

Since the studies by Armanini and coworkers, it appeared that PHA1 was due to a tubular insensitivity to aldosterone action. After the cloning of the MR complementary DNA sequence in 1987 (Arriza *et al.* 1987), early studies failed however to identify MR mutations in one family with autosomal dominant inheritance (Zennaro *et al.* 1994) and in the index PHA1 case described by Cheek and Perry (Komesaroff *et al.* 1994). No MR abnormalities were also found in one case with generalized PHA, where the authors postulated a defect in a post-MR step of aldosterone action (Arai *et al.* 1994). It was only in 1998 that Geller and coworkers confirmed the initial hypothesis of a primary defect in the MR, by identifying two frameshift mutations, two premature termination codons and one splice donor mutation in the MR in four dominant and one sporadic cases of PHA1 (Geller *et al.* 1998). The main difference between this and previous studies was the technical approach for detecting mutations. Indeed, after the cloning of the *NR3C2* gene (Zennaro *et al.* 1995), genomic sequencing became available allowing to detect mutations directly on DNA. A p.Arg590X MR nonsense mutation was subsequently detected in affected patients from the kindred studied by Zennaro and coworkers, which was not present on MR mRNA in peripheral blood lymphocytes from the same patient (Geller *et al.* 2006). This is likely a consequence of nonsense-mediated mRNA decay, whereby mRNA carrying premature stop codons in non-terminal exons is degraded and suggests that MR haploinsufficiency is sufficient to cause renal PHA1. Sequencing of the coding exons and intron–exon junction has eventually identified the causal mutation of the Australian index case of PHA1 also, who was carrier of a p.Leu938Arg mutation transmitted from his asymptomatic mother (Fuller *et al.* 2011).

Subsequently, more than fifty different MR mutations have been reported in the literature (Arai & Chrousos 2000, Geller 2005, Riepe 2009, Zennaro *et al.* 2012). Disease-causing mutations are located in all exons of the *NR3C2* gene and affect all functional domains of the MR. The human MR is a protein of 984 amino acids with a modular structure comprising three separate domains with specific functions (Viengchareun *et al.* 2007, Yang & Young 2009). The *NR3C2* gene is composed of 8

coding exons (Zennaro *et al.* 1995); exon 2 codes for the N-terminal domain (NTD), involved in transcriptional activation and intramolecular interactions, whereas exons 3 and 4 code for the DNA-binding domain (DBD). This central domain of the protein folds into two zinc-finger structures that are involved in DNA recognition and receptor dimerization (Hudson *et al.* 2014). Exons 5–9 code for the ligand-binding domain (LBD), which also contains regions involved in interaction with heat shock proteins, transcriptional activation and dimerization (for a detailed review see Pascual-Le Tallec & Lombes 2005, Huyet *et al.* 2012, Fuller 2015). This region harbours a ligand-dependent activation function AF2. Remarkably, to date, only nonsense, frameshift or splice-site mutations have been identified in exon 2. Although functional common single-nucleotide polymorphisms have been identified in this region (DeRijk *et al.* 2006, see below), it appears that changes in MR function supported by the N-terminus are not translating into a sufficiently severe salt-losing disorder to be diagnosed as PHA1. This is probably due to the specific conformation of the domain, which is supposed to be unstructured in solution, undergoing induced folding upon DNA and/or protein binding (Lavery & McEwan 2005).

The functional consequences of certain mutations located in the DBD and the LBD have been studied *in vitro* for their effects on hormone binding, DNA binding and transcriptional activation. LBD mutations diminish or completely abolish ligand binding, whereas DBD mutations affect basal or dynamic DNA binding and eventually intracellular trafficking. In some cases, those effects could be predicted on the basis of previous experimental evidence and the crystal structure of the MR LBD (Fagart *et al.* 2005, Pujo *et al.* 2007, Huyet *et al.* 2012). Conversely, in some cases, MR mutations identified in PHA1 patients have allowed the identification of crucial amino acids involved in given receptor functions (Sartorato *et al.* 2003, 2004a). The MR LBD domain contains 11 helices (H1 and H3–H12) and two short β -sheets organized in the three helical sandwich folds common to all members of the nuclear receptor family (Fig. 1) (Bledsoe *et al.* 2005, Fagart *et al.* 2005, Li *et al.* 2005). This scaffold delimits a binding pocket at the lower part of the domain. Twenty-two residues of the H3, H5, H7, H11 and H12 helices, the β -strand between the H5 and H6 helices and the loop between the H11 and H12 helices form the ligand-binding cavity of the MR (Fagart *et al.* 1998). At one extremity of the ligand-binding cavity, Gln776 (H3 helix) and Arg817 (H5 helix) establish hydrogen bonds with the 3-ketone function, which characterizes

all the MR-binding steroids. At the other extremity of the ligand-binding pocket, Thr945 (H11 helix) is in contact with the 20-ketone function of steroids and Asn770 with the 21-hydroxyl group of MR agonists. As a consequence, mutations affecting amino acids 770 and 776 have major consequences on aldosterone binding and transcriptional activation by the MR (Sartorato *et al.* 2004a, Pujo *et al.* 2007). On the other hand, analysis of a p.Leu979Pro mutation identified in PHA1 has allowed the identification of hydrophobic interactions in the extreme C-terminal tail of the MR that are essential for establishing the ligand-binding competent state of the receptor (Sartorato *et al.* 2004a).

In addition to haploinsufficiency, due to complete loss-of-function or nonsense-mediated mRNA degradation, MR-carrying mutations may also exert dominant negative effects on the wild-type receptor. This is because MR binds as a dimer on hormone-responsive elements in the regulatory regions of target genes. In this case, the effects are strongly promoter dependent, and functional consequences associated with MR mutations may be distinct on different genes and in different tissues (Sartorato *et al.* 2004b). Indeed, investigation of four MR mutations affecting residues in the LBD identified in families with PHA1 supports this view. Measurement of aldosterone-dependent gene expression of endogenous *sgk1*, *GILZ*, *NR3C2* and *SCNN1A*, four bona fide MR target genes with different kinetic responses, in renal cell has shown that different MR mutants with comparable loss of ligand binding differently affect individual gene expression profiles (Fernandes-Rosa *et al.* 2011). In particular, although receptors carrying a p.Leu848Pro and p.Arg947Ter mutation were incapable of inducing *sgk1*, *GILZ*, *NR3C2*, and *SCNN1A* expression, receptors carrying a p.Ser843Pro mutation retained complete transcriptional activity on *sgk1* and *GILZ* gene expression, and MR carrying a p.Leu877Pro mutation negatively affected the expression of *sgk1*, *NR3C2* and *SCNN1A*. The gene-dependent differences in transcriptional activity of mutant receptors might be explained by different promoter organizations and by modifications in the three-dimensional structure of the MR LBD, which may differentially affect MR interactions with target promoters and the set of transcriptional coregulators recruited to the receptor. These studies suggest that not only the extent of functional reduction but also the specific qualitative loss of function, in terms of regulated gene expression, modulate the phenotype in PHA1 and may constitute the mechanistic substrate for phenotypic variability of the disease (Fernandes-Rosa *et al.* 2011).

10 years of genetic diagnosis of PHA1

The genetics laboratory at the Hôpital Européen Georges Pompidou (HEGP) is the French referral center for the genetic diagnosis of PHA1, which is part of the reference network MARHEA for hereditary kidney diseases of the child and adult (www.sfndt.org/sn/marhea/) and is accredited to ISO 15189 standard by the relevant French authority. 236 affected patients have been assessed for genetic diagnosis in our laboratory since 2004. Improved case detection together with genetic analysis has allowed to identify ≈100 *NR3C2* mutations or exon deletions in patients with renal PHA1 and more than 30 mutations in *SCNN1A*, *SCNN1B* or *SCNN1G* in generalized PHA1 and to discover a continuum of phenotypically and/or biologically distinct forms of PHA1 (Dirlewanger *et al.* 2011, Hubert *et al.* 2011). Genetic diagnosis is performed by targeted Sanger sequencing, based on clinical and biochemical characteristics; more recently, development of a targeted NGS kit has allowed simultaneous sequencing of *NR3C2*, *SCNN1A*, *SCNN1B* and *SCNN1G* in referred patients and better characterization of cases with diagnostic ambiguity.

NR3C2 mutations were found in 62% of patients with renal PHA1 (Table 1). In 61 cases, the mutation was inherited from one of the parents, in 24 cases from the father and in 37 cases from the mother. *De novo* mutations were found in 20 cases, and in 17 cases, transmission is unknown because the parents were not investigated. It is important to note that in many familial cases, clinical diagnosis was suggestive of a sporadic form with no family history, and genetic diagnosis allowed identification of asymptomatic carriers and subsequent genetic counseling. In particular, genetic diagnosis can be performed on cord blood, allowing for rapid salt supplementation if required and prevention of severe dehydration of newborns in affected families. The exact causes of intrafamilial phenotypic heterogeneity in renal PHA1 are unknown, but intercurrent events, such as infections, vomiting or diarrhea may worsen an otherwise subclinical neonatal salt loss in individuals at risk. Naturally occurring hypomorphic or hyperfunctioning alleles of other genes, coding for proteins involved in distal sodium reabsorption, may also modulate the phenotype.

Twenty mutations were found in exon 2; all of them lead to truncated receptors. Of the 22 mutations identified in exons 3 and 4, coding for the MR DBD, 11 were nonsense or frameshift mutations, the reminder missense mutations. Thirty variants were located in exons 5–9 and affected the LBD; the majority were missense mutations,

Table 1 Summary of MR mutations identified in PHA1 patients at the genetics laboratory of the HEGP.

Kindred	Mutation	Protein	Exon	MR domain	Transmission	References
K158	c.415C>T	p.Gln139Ter	2	NTD	<i>De novo</i>	
K074	c.497_498delCT	p.Ser166Ter	2	NTD	Father	Hubert et al. 2011
K096_1	c.497-498delCT	p.Ser166Ter	2	NTD	Father	Hubert et al. 2011
K096_4	c.[497-498delCT];[2418G>A]	p.[Ser166Ter];[Trp806Ter]	2/6	NTD/LBD	Mother/ father	Hubert et al. 2011
K161	c.531dupG	p.Arg178Alafs*5	2	NTD	<i>De novo</i>	
K306	c.556_557del	p.Met186Valfs*3	2	NTD	Mother	
K066	c.887-888delCT	Ser296Cysfs*3	2	NTD	Father	
K390	c.981delC	p.Ser328Valfs*10	2	NTD	<i>De novo</i>	Pujo et al. 2007
K440	c.1029 C>A	p.Tyr343Ter	2	NTD	Mother	Pujo et al. 2007
K115	c.1108C>T	p.Gln370Ter	2	NTD	Father	
K261	c.1108C>T	p.Gln370Ter	2	NTD	<i>De novo</i>	
K155	c.1169delG	p.Gly390Alafs*9	2	NTD	<i>De novo</i>	
K128	c.1213delG	p.Ala405Leufs*7	2	NTD	Father	
K043	c.[1237G>A; 1271C>G]	p.[(Gly413Arg ;p. Ser424Ter)]	2	NTD	Mother	
K061	c.1380delT	p.Phe460Leufs*14	2	NTD	Mother	
K087	c.1506T>G	p.Tyr502Ter	2	NTD	Mother	
K235	c.1609C>T	p.Arg537Ter	2	NTD	Father	Geller et al. 1998
K136	c.1672G>T	p.Glu558Ter	2	NTD	?	
K025	c.1679G>A	p.Trp560Ter	2	NTD	Mother	Pujo et al. 2007
K251	c.1698del	p.Ser567Argfs*11	2	NTD	Father	
K109	(c.299-?)_(c.2510+1_2511-1)del		Del exon 2-6		Father	
K091	(c.229-?_c.*2955+?)del		Del exon 2-9		<i>De novo</i>	
K127	(c.229-?_c.*2955+?)del		Del exon 2-9		<i>De novo</i>	
K241	(c.229-?_c.*2955+?)del		Del exon 2-9		?	
K047	c.1757+1G>A	Splice	Intron 2		<i>De novo</i>	Belot et al. 2008
K104	c.1757+1G>A	Splice	Intron 2		Mother	Belot et al. 2008
K110	c.1757+1G>C	Splice	Intron 2		Mother	
K032	c.1768C>T	p.Arg590Ter	3	DBD	Father	Geller et al. 2006
K238	c.1768C>T	p.Arg590Ter	3	DBD	<i>De novo</i>	Geller et al. 2006
K292	c.1790C>G	p.Ser597Ter	3	DBD	<i>De novo</i>	
K080	c.1807T>C	p.Cys603Arg	3	DBD	Father	
K063	c.1808G>C	p.Cys603Ser	3	DBD	Mother	
K089	c.1808G>C	p.Cys603Ser	3	DBD	Father	
K018	c.1811delT	p.Leu604trpfs*13	3	DBD	Mother	Pujo et al. 2007
K059	c.1817G>C	p.Cys606Ser	3	DBD	Mother	Loomba- Albrecht et al. 2010
K090	c.1834G>T	p.Gly612Ter	3	DBD	Father	
K218	c.1867T>C	p.Cys623Arg	3	DBD	Mother	
K273	c.1868-1869delinsTT	p.Cys623Phe	3	DBD	Mother	
K291	c.1897G>A	p.Gly633Arg	3	DBD	?	Sartorato et al. 2003
K257	c.1916G>A	p.Cys639Tyr	3	DBD	?	
K078	c.1951C>T	p.Arg651Ter	3	DBD	Mother	Derache et al. 2012
K014	c.(1757+1_1758-1)_ (1897+1_1898-1)del	p.Ser586Argfs*12	Del exon 3		<i>De novo</i>	
K016	c.(1757+1_1758-1)_ (1897+1_1898-1)del	p.Ser586Argfs*12	Del exon 3		?	
K033	c.(1757+1_1758-1)_ (1897+1_1898-1)del	p.Ser586Argfs*12	Del exon 3		Mother	
K119	c.(1757+1_1758-1)_ (1897+1_1898-1)del	p.Ser586Argfs*12	Del exon 3		Father	

(Continued)

Table 1 Continued.

Kindred	Mutation	Protein	Exon	MR domain	Transmission	References
K164	c.(1757+1_1758-1)_ (1897+1_1898-1)del	p.Ser586Argfs*12	Del exon 3		Mother	
K137	c.(1757+1_1758-1)_ (2014+1_2015-1)del	p.Ser587Thr*71	Del exon 3-4		Father	
K079	c.(1757+1_1758-1)_ (2510+1_2511-1)del	p.Ser586_Glu837delinsArg	Del exon 3-6	DBD/LBD	Mother	
K106	c.1912_1915del	p.Leu638Valfs*30	4		Mother	
K012	c.1934G>C	p.Cys645Ser	4	DBD	Mother	Pujo et al. 2007
K021	c.1935C>A	p.Cys645Ter	4	DBD	De novo	Sartorato et al. 2003
K001	c.1954C>T	p.Arg652Ter	4	DBD	Mother	Pujo et al. 2007
K011	c.1954C>T	p.Arg652Ter	4	DBD	?	Pujo et al. 2007
K055	c.1954C>T	p.Arg652Ter	4	DBD	De novo	Pujo et al. 2007
K296	c.1955G>A	p.Arg652Gln	4	DBD	?	
K034	c.1977A>C	p.Arg659Ser	4	DBD	Father	Pujo et al. 2007
K008	c.(1897+1_1898-1)_ (2014+1_2015-1)del	p.Gly633_Gly671del	Del exon 4	DBD	Mother	
K219	c.(1897+1_1898-1)_ (2014+1_2015-1)del	p.Gly633_Gly671del	Del exon 4	DBD	Father	
K188	(c.1897+1_1898-1)_ (c.2799+1_2800-1)del	p.Gly633Alafs*80	Del exon 4-8		De novo	
K099	(c.1897+1_1898-1)(c.*2955+?)del	p.Gly633_Lys984del	Del exon 4-9	DBD/LBD	De novo	
K041	c.2020A>T	p.Lys674Ter	5	LBD	Father	Pujo et al. 2007
K064	c.2145delA	p.Glu716Asnfs*57	5	LBD	?	
K275	c.2194C>T	p.Arg732Ter	5	LBD	Mother	
K092	c.2270C>G	p.Ser757Ter	5	LBD	?	
K013	c.2275C>T	p.Pro759Ser	5	LBD	Father	Pujo et al. 2007
K308	c.2296C>G	p.Leu766Val	5	LBD	Father	
K026	c.2306_07invTC	p.Leu769Pro	5	LBD	De novo	Pujo et al. 2007
K133	c.2309A>G	p.Asn770Ser	5	LBD	Mother	
K138	c.2309A>G	p.Asn770Ser	5	LBD	Mother	
K030	c.2310C>A	p.Asn770Lys	5	LBD	Father	Pujo et al. 2007
K159	c.2347T>C	p.Trp783Arg	5	LBD	Mother	
K024	c.(2014+1_2015-1)_ (2799+1_2800-1)del	p.Arg673Glyfs*79	Del exon 5-8		Mother	
K239	(c.2014+1_2015-1)_ (c.2799+1_2800-1)del	p.Arg673Glyfs*79	Del exon 5-8		Mother	
K167	c.2365+2T>C	Splice	Intron 5		De novo	
K288	c.2365+1G>A	Splice	Intron 5		?	
K015	c.2413 T>C	p.Ser805Pro	6	LBD	Mother	Pujo et al. 2007
K096_2	c.[2418G>A]	p.Trp806Ter	6	LBD	Mother	Hubert et al. 2011
K031	c.2445C>A	p.Ser815Arg	6	LBD	Mother	Pujo et al. 2007
K165	c.2453C>T	p.Ser818Leu	6	LBD	Father	Geller et al. 2006
K168	c.2453C>T	p.Ser818Leu	6	LBD	?	Riepe et al. 2006
K027	c.(2365+1-2366-1)(c.2955+?)del	p.Gly789_Lys984del	Del exon 6-9		De novo	Geller et al. 2006
K060	c.2527T>C	p.Ser843Pro	7	LBD	Mother	Fernandes-Rosa et al. 2011
K131	c.2533A>G	p.Met845Val	7	LBD	?	
K300	c.2534_2561dup	p.Gln854Hisfs*3	7	LBD	De novo	
K221	c.2545T>C	p.Cys849Arg	7	LBD	Mother	
K268	c.2581C>T	p.Arg861Ter	7	LBD	Father	Uchida et al. 2009
K054	c.2630T>C	p.Leu877Pro	7	LBD	Father	Fernandes-Rosa et al. 2011
K246	c.2657T>G	p.Leu886Arg	8	LBD	?	

(Continued)

Table 1 Continued.

Kindred	Mutation	Protein	Exon	MR domain	Transmission	References
K083	c.2753G>A	p.Trp918Ter	8	LBD	Mother	
K280	c.2758_2759dup	p.Phe921Glyfs*5	8	LBD	?	
K294	c.2766C>G	p.Tyr922Ter	8	LBD	?	
K019	c.2799+1G>A	Splice	Intron 8		<i>De novo</i>	Pujo <i>et al.</i> 2007
K023	c.2799+1G>A	Splice	Intron 8		Mother	Pujo <i>et al.</i> 2007
K062	c.2799+1G>A	Splice	Intron 8		Father	Pujo <i>et al.</i> 2007
K304	c.2799+1G>A	Splice	Intron 8		?	Pujo <i>et al.</i> 2007
K298	c.2799+1G>A	Splice	Intron 8		Mother	Pujo <i>et al.</i> 2007
K102	c.2813T>G	p.Leu938Arg	9	LBD	Mother	Fuller <i>et al.</i> 2011
K140	c.2900_2903dup	p.Pro969Alafs*46	9	LBD	<i>De novo</i>	
K266	(c.2799+1_2800–1)_(c.*2955+?)del	p.leu934_Lys984del	Del exon 9	LBD	?	

underscoring the importance of individual amino acids in the ligand-binding domain for ensuring an appropriate conformation of the MR ligand-binding pocket. Nine splice variants were identified in different introns. In patients with renal PHA1, in whom no *NR3C2* mutation was detected, large deletions were searched for by SNP analysis in informative families and by quantitative multiplex PCR of short fluorescent fragments (qMPSF). Large deletions encompassing single or multiple exons and the flanking intronic regions of the *NR3C2* gene were identified in 19 of 160 patients with renal PHA1 (12%), confirming the previous results suggesting that large deletions of the *NR3C2* gene are reasonably frequent in PHA1 patients (Pujo *et al.* 2007).

Remarkably, one of our patients was compound heterozygous carrier of two different nonsense mutations of the MR, p.[Ser166Ter];[Trp806Ter], representing the first case of severe recessive renal PHA1 caused by MR mutations (Hubert *et al.* 2011). In this family, the index case was diagnosed with PHA1, and genetic screening identified a frameshift mutation in exon 2 (c.497_498delCT) resulting in a premature stop codon at amino acid position 166 (p.Ser166Ter) (Table 1, K096_1). The family history indicated that three other members were possibly affected by PHA1. Familial genetic screening for the Ser166X mutation revealed that the mutation was transmitted from the father to the index case and was also present in one sibling, patient K096_4 (Table 1), but absent in the other affected sibling. The parents of the family subsequently participated in the clinical trial 'PHACARV – Cardiovascular Evaluation of Adult PHA 1 Patients' (Escoubet *et al.* 2013); both father and mother had a similar phenotype suggesting PHA1 with increased plasma aldosterone levels. This unexpected result prompted us to sequence the entire *NR3C2* coding sequence and intron–exon junctions in the asymptomatic mother. A missense

mutation was found in exon 6 (c.2418G>A) replacing tryptophan at amino acid position 806 with a stop codon. Genetic screening for this second mutation in the pedigree revealed that the mutation had been transmitted to the asymptomatic patient K096_2 (Table 1). Patient K096_4 was compound heterozygous for both familial mutations. Analysis of MR expression and residual MR function showed that both alleles were expressed in lymphocytes from affected carriers, but *ex vivo* experiments indicated that MR carrying the p.Ser166Ter mutation was degraded. MR carrying the p.Trp806Ter mutation completely lost aldosterone binding, but showed minimal constitutive ligand-independent transcriptional activity. Altogether, these results suggested that the PHA1 phenotype resulted from combined haploinsufficiency and partial loss of function. This exceptional case demonstrates that minimal residual activity of MR is compatible with life. It also suggests that rare hypomorphic *NR3C2* alleles may be more common than expected from the prevalence of detected PHA1 cases and may affect renal salt handling and blood pressure in the general population.

An activating MR mutation in a rare Mendelian form of arterial hypertension

By screening for *NR3C2* in 75 patients with early-onset severe hypertension, Geller and coworkers identified a heterozygous p.Ser810Leu mutation in a 15-year-old boy with severe hypertension associated with suppressed plasma renin and low aldosterone levels (Geller *et al.* 2000). Screening of the family of the index case identified 11 relatives that had been diagnosed with severe hypertension before age 20 years, thus defining a new Mendelian form of arterial hypertension. Remarkably, two female carriers of the mutation had all their pregnancies complicated by

marked exacerbation of hypertension, accompanied by low serum potassium levels and undetectable aldosterone levels, but without signs of preeclampsia.

The MR p.Ser810Leu mutation lies in the MR ligand-binding pocket. It induces a major change in the LBD conformation, leading to antagonist ligands switching to become agonists. Functional studies in cells have shown that although the transcriptional activity of wild-type and mutant MR were indistinguishable in response to aldosterone, progesterone and its derivatives all activated transcription via MR carrying the p.Ser810Leu mutation. Spironolactone, which is clinically used as MR antagonist, was also a potent agonist on the mutant receptor. Structural analysis of the MR ligand-binding pocket via 3D homology models and later through established X-ray crystal structures has explained the structural determinants of these changes in ligand properties. Under normal circumstances agonist ligands, such as aldosterone, stabilize the active conformation of the LBD by anchoring of their 21-hydroxyl function on Asn770 of helix 3 of the MR (see above). Antagonist ligands such as progesterone and RU26752 are unable to establish this contact (Fagart *et al.* 1998). The antagonist behavior of spironolactones has been explained by a clash between the Ala773 residue and substitutions at the 11 β position in spironolactones, which are expected to hinder their accommodation in the ligand-binding pocket. Structural and biochemical studies indicated that the p.Ser810Leu mutation results in the gain of a van der Waals interaction between helix 5 and helix 3 that substitutes for interaction of the steroid 21-hydroxyl group with helix 3 in the wild-type receptor, thus stabilizing the receptor in an active conformation.

The first MR LBD X-ray crystal structure to be reported was that of the mutant MR carrying the p.Ser810Leu mutation (Fagart *et al.* 2005) complexed with deoxycorticosterone (DOC) and progesterone, which both act as agonists on the mutated receptor. The most striking observation was that the Leu810 residue (in helix 5) establishes hydrophobic contacts with the Gln776 residue (helix H3) and with the 19-methyl group of both DOC and progesterone.

In the presence of these contacts, contact between the ligand and the Asn770 residue is no longer required, and antagonist ligands that do not contact the Asn770 residue in the wild-type MR are able to stabilize MRS810L in its active conformation (Fagart *et al.* 2005).

In subsequent studies, Rafestin-Oblin and coworkers have identified cortisone and 11-dehydrocorticosterone as being the endogenous ligands responsible for constitutive

MR activation and hypertension in men and non-pregnant women carrying the MR p.Ser810Leu mutation (Rafestin-Oblin *et al.* 2003). Those steroids are the main metabolites of cortisol and corticosterone produced by the action of the 11HSD2. In contrast with their low affinity for the wild-type MR, cortisone and 11-dehydrocorticosterone bind MRS810L with high affinity, leading to its activation and induction of MR-dependent transcriptional activation. As plasma concentration of cortisol is about 30-fold higher than that of corticosterone, it is likely that cortisone triggers most of the phenotype in affected carriers.

Despite intensive search of the MR p.Ser810Leu mutation in patients with early-onset hypertension and/or pregnancy-induced hypertension (unpublished data), no further family has been reported in the literature. However, the variant is not described in exome data from the ExAC (<http://exac.broadinstitute.org/>), which has collected data from >60,000 unrelated individuals sequenced as part of various disease-specific and population genetic studies (Lek *et al.* 2016). It therefore remains to be established whether this remains a textbook case from which we have learned enormously in terms of structure–function relationships of the MR, or whether it might be relevant for hypertensive pregnant women in a larger context. Given the functional characteristics of MR carrying the p.Ser810Leu mutation, spironolactone, as well as eplerenone, cannot be used to treat hypertension in these patients (Hultman *et al.* 2005). In contrast, both BR-4628 and finerenone, two MR antagonists derived from the chemical class of dihydropyridines, are potent MR antagonists that retain their antagonist character at the MRS810L mutant (Fagart *et al.* 2010, Amazit *et al.* 2015). As finerenone is currently developed for clinical use and has been studied in different clinical trials, it might be a therapeutic option to treat patients carrying the MR p.Ser810Leu mutation.

Influence of MR gene variants on blood pressure, stress and the HPA axis

In addition to rare MR variants, frequent *NR3C2* polymorphisms have been shown to exert quantitative effects on MR function and to modulate salt balance, blood pressure, stress and the hypothalamic–pituitary–adrenal (HPA) axis. The *NR3C2* polymorphism c.-2G>C (rs2070951, MAF $\approx$ 0.45) is a frequent single-nucleotide polymorphism located in the 5'-untranslated region of the *NR3C2* gene, 2 nucleotides upstream of the first translation start site. *In vitro* characterization has revealed

that the G allele is associated with decreased MR protein levels and reduced transcriptional activation compared to the C allele both in the presence of aldosterone and cortisol. In different groups of patients, the G allele of the c.-2G>C polymorphism is associated with increased activation of the renin–angiotensin–aldosterone axis and with increased blood pressure, probably related to decreased MR expression. Subjects with the GG genotype had significant higher plasma renin levels both in a mild hypertensive group subjected to a salt sensitivity test and in a healthy normotensive group included in a crossover study to receive both a high and low Na/K diet compared to homozygous C carriers; the GG genotype was also correlated with higher plasma aldosterone levels in healthy subjects. In mild hypertensives and in a large cohort for depression and anxiety, the genotype GG was associated with higher systolic blood pressure in males. These studies provide evidence that frequent polymorphisms of the MR may exert quantitative effects on the activity of the renin–angiotensin–aldosterone axis and blood pressure in the general population, modulating vulnerability for hypertension (van Leeuwen *et al.* 2010).

In addition to its function in maintaining salt homeostasis and regulating blood pressure, the MR also plays a role in the brain, where it regulates salt appetite, blood pressure, stress response and cognitive processes (Gomez-Sanchez *et al.* 1990, de Kloet *et al.* 2000; for a detailed review, see the article by Joëls and de Kloet in this issue). In the brain, MR is expressed in limbic regions, particularly in hippocampal neurons; in the absence of 11HSD2, the brain MR is essentially occupied and activated by cortisol. Coordinately with the glucocorticoid receptor the MR regulates the onset and termination of the stress response. Central MR is also involved in the control of autonomic outflow as demonstrated in rats by modulation of stress-induced heart rate and blood pressure responses through central application of MR antagonists (de Kloet *et al.* 2000).

Several studies performed by the group of Roel de Rijk and coworkers have explored the effects of common MR variants on stress response and depression. By studying the association between the common *NR3C2* single-nucleotide polymorphism c.538A>G/p.Ile180Val (rs5522, MAF 0.12) and outcome variables in a healthy cohort subjected to psychosocial challenge (Trier Social Stress Test, TSST), it was shown that carriers of the MR180Val allele had higher plasma and saliva cortisol levels and higher heart rate responses to the TSST than non-carriers (DeRijk *et al.* 2006). In contrast, there was no difference in salt sensitivity according to genotypes

in a group of mild hypertensives undergoing a controlled sodium diet followed by a salt sensitivity test in terms of blood pressure, cardiac frequency and renal sodium handling. The differences in central and renal MR effects were explained by the functional consequences of the p.Ile180Val variant on receptor function. Although aldosterone-mediated transactivation of different reporter genes was undistinguishable, whatever the amino acid at position 180, in the presence of cortisol, the transcriptional activity of MR180Val was significantly lower than that of the MR180Ile. This suggests that p.Ile180Val polymorphism, which is located in the NTD, may affect the intramolecular interactions within the MR or binding of specific coactivators and modulate specifically cortisol-mediated MR effects.

In addition to p.Ile180Val and c.-2G>C, exonic sequencing of *NR3C2* in fifty individuals from the Dutch population resulted in the identification of two major haplotype blocks, one in the 5'-region (based on p.Ile180Val and c.-2G>C) and one in the 3'-region (ter Heegde *et al.* 2015). One haplotype in particular, associating c.-2C and p.Ile180 with SNPs in the promoter region of the *NR3C2* gene has a frequency of 0.38 in the general population. In addition to increased MR protein expression and differences in transcriptional responses related to c.-2C and p.Ile180, the SNPs in the promoter region are associated with higher promoter activity, resulting in enhanced MR activity (Klok *et al.* 2011). Haplotype 2 was associated, in women, with heightened dispositional optimism in a cohort of elderly subjects, with less hopelessness and rumination in a cohort of 150 university students and with a lower risk of depression in a large genome-wide association study. These and other studies suggest that common functional MR haplotypes are important determinants of inter-individual variability in basal and stress-induced HPA axis activity and stress-related appraisal and learning, by differentially mediating cortisol effects on different systems (ter Heegde *et al.* 2015). Remarkably, in adult PHA1 patients carrying MR loss-of-function mutations, personal history revealed an increase in depression compared to paired non-carriers (Escoubet *et al.* 2013).

Study of adult PHA1 patient carriers of MR mutations has also allowed definition of the contribution of MR to negative feedback of the HPA axis in humans (Walker *et al.* 2014). In a study performed on a subset of patients from the cohort described in Escoubet and coworkers (Escoubet *et al.* 2013), patients with MR mutations had higher morning plasma cortisol and increased 24-h urinary excretion of cortisol, independent of gender. Higher

plasma cortisol was associated with higher plasma renin, lower serum high-density lipoprotein cholesterol and higher waist circumference but not with blood pressure, carotid intima-media thickness or echocardiographic parameters. These data suggest that hypercortisolemia is related to the severity of MR deficiency and has features of glucocorticoid excess mediated by glucocorticoid receptors on liver lipid metabolism and adipose tissue distribution, without adversely affecting cardiac and vascular remodeling in the absence of normal signaling through the MR (Walker *et al.* 2014).

Concluding remarks

The MR cDNA was cloned by Arriza and coworkers in 1987 (Arriza *et al.* 1987), followed by the determination of the human mineralocorticoid receptor gene structure and identification of expressed isoforms (Zennaro *et al.* 1995), the characterization of two different promoters in the human mineralocorticoid receptor gene 5'-regulatory region (Zennaro *et al.* 1996) and the identification of different human MR splice variants, some with particular functional properties possibly modulating corticosteroid effects in target tissues (Zennaro *et al.* 2001). It appeared from these and other data in rats and mice that the *NR3C2* gene undergoes different levels of regulation, leading to tissue and developmentally regulated MR protein expression of different receptor isoforms. Additional mechanisms of hormone selectivity and transcriptional coregulator recruitment contribute to specific and multiple sets of physiological responses. In addition, recent genome-wide mapping of MR binding on regulatory sites of target genes has further highlighted the complexity of transcriptional regulation exerted by the MR (Le Billan *et al.* 2015). As a result, MR mutations identified in PHA1 not only differentially affect gene expression profiles in a promoter-dependent manner (Fernandes-Rosa *et al.* 2011), but may also have different consequences depending on target tissues, developmental stage and MR ligand, which may explain the heterogeneous phenotypic expression of the disease, even within the same family, and the absence of signs of mineralocorticoid resistance outside the kidney in renal PHA1. Remarkably, in the context of reduced MR function, high salt and high aldosterone do not exert deleterious effects on the cardiovascular system (Escoubet *et al.* 2013), pointing to a central role of the receptor in aldosterone- or glucocorticoid-mediated end-organ damage. It is particularly good news, 30 years after its cloning, that the

MR is not only responsible for maintaining blood pressure and salt homeostasis, but that a good MR function may contribute to enhance optimism and protect against depression (although in females only!).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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